bio-protocol Preprint

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Confocal microscopy and image analysis

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An abbreviated version of this protocol was published in eLIFE in Jun 2020

Nanobody-directed targeting of optogenetic tools to study signaling in the primary cilium

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Detailed protocol

The settings applied in the CiliaQ workflow are not directly transferable from one to another data set, when a different microscope, different microscope settings and/or a different cilia-labeling approach has been applied. To perform a CiliaQ analysis on your data set, please read the CiliaQ publication for more information and analysis examples (https://link.springer.com/article/10.1140/epje/s10189-021-00031-y) and the User guide for how to perform the analysis workflow (https://github.com/hansenjn/CiliaQ). To establish settings for your data set, you may also have a look at our instructions and tutorials made accessible in the wiki of the CiliaQ Github repository (https://github.com/hansenjn/CiliaQ/wiki).

The general protocol for analyzing cilia length is:

1. Record 3D images with a confocal microscope: z step size of about 0.5 µm, 60x oil-immersion objective, zoom-factor 1x or higher, 1024x1024 pixel or higher (resulting in a pixel size of maximum 0.25 µm, ideally use a lower pixel size).
2. Segment cilia from background with CiliaQ Preparator (here you need to first establish settings for your data set – see the according tutorials in the CiliaQ wiki https://github.com/hansenjn/CiliaQ/wiki)
3. Use CiliaQ Editor to manually inspect the segmentation results and correct the mask of cilia that were incompletely or incorrectly detected.
4. Apply CiliaQ to quantify the edited images
   a. For length quantification, you need to particularly optimize the CiliaQ setting “Gauss filter XY sigma” for your data set, because it controls the accuracy of the length detection – see CiliaQ publication, Fig. 1 F,G (https://link.springer.com/article/10.1140/epje/s10189-021-00031-y/figures/1).
5. Retrieve the length from CiliaQ output files

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hansen, J. , Kaiser, F. , Schmidt, F. I. and Wachten, D. (2021). Confocal microscopy and image analysis. Bio-protocol Preprint. bio-protocol.org/prep1125.
2. Hansen, J. N., Kaiser, F., Klausen, C., Stüven, B., Chong, R., Bönigk, W., Mick, D. U., Möglich, A., Jurisch-Yaksi, N., Schmidt, F. I. and Wachten, D.(2020). Nanobody-directed targeting of optogenetic tools to study signaling in the primary cilium. eLIFE. DOI: 10.7554/eLife.57907

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